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Aminocarbazole alkaloid derivatives as potential modulators of p53mutants

Solida Long[1], Nanthicha Thongdee[2], Joana B. Loureiro[3], Madalena M. Pinto[1], Ploenthip Puthongking[2], Lucília Saraiva[3], Emilia Sousa[1*]

[1] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal & CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal
[2] Faculty of Pharmaceutical Sciences, Khon Kaen University, 40002, Thailand
[3] UCIBIO/REQUIMTE, Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

Overview: The tumor suppressor protein p53 is a major regulator of key cellular processes, including cell cycle arrest, apoptosis, DNA repair, stemness, invasion and migration. *TP53* is the most commonly mutated gene in human cancer. In fact, over half of human cancers contain p53 mutations, particularly missense mutations, preferentially localized within the p53 DNA-binding domain (Figure 1). Mutant p53 are frequently associated with patient poor prognosis due to more aggressive tumor phenotypes, particularly increased proliferation, metastasis and resistance to therapy [1]. Heptaphylline is a carbazole alkaloid, isolated from *Clausena harmandiana* with promising antitumor effects. Herein, the semisynthesis of heptaphylline and two related secondary metabolites was performed for the purposes of enhancing their antitumor activity and improve physico-chemical properties. The molecular modifications were based on introduction of amine derivative moieties, which are present in some known p53 modulators.
Reductive aminations using sodium triacetobohydride in acidic conditions (Scheme 1) were applied on three natural isolated carbaldehyde carbazole alkaloids to obtain a small library of amino derivatives (Figure 2) [2]. Both natural isolated carbazoles and amino derivatives were tested for their ability to inhibit tumor cell growth in human tumor cell lines expressing different forms of p53 (wild-type, mutants). Moreover, using a yeast-screening assay [3], it was investigated the ability of heptaphylline to reactivate several mutant p53 forms with high prevalence in human cancer (Table 1). Natural occuring carbazoles and the first series of amines investigated showed promising tumor cell growth inhibitory effects with GI_{50}<10μM (Figure 3). Also, some derivatives reestablished the wild-type-like activity to several mutant p53 in yeast, behaving as potential reactivators of mutant p53. These results suggest the discovery of a potential lead compound for the development of anticancer agents p53 modulators.

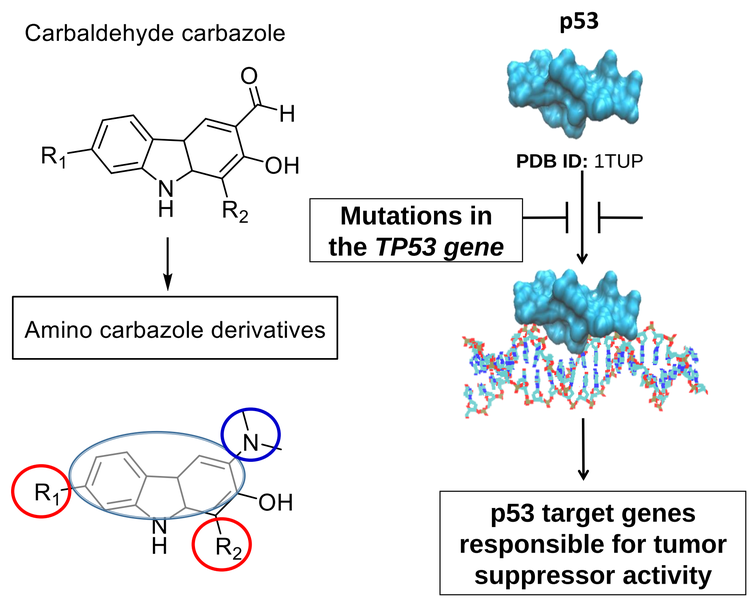
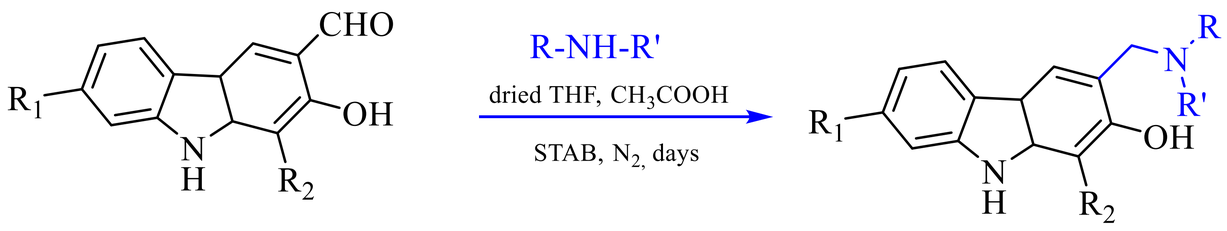


Figure 1 : The regulation of p53.

Methods:



1. R_1= H, R_2=$CH_2CH(CH_3)_2$, heptaphylline
2. R_1= OCH_3, R_2=$CH_2CH(CH_3)_2$, 7-methoxy heptaphylline
3. R_1= OCH_3, R_2=H , 2-hydroxy-3-formyl-7-methoxy-carbazole

Scheme 1: Reductive amination of carbadehyde carbazoles with amine precursors. THF = Tetrahydrofuran, STAB = Sodium triacetoxyborohydride

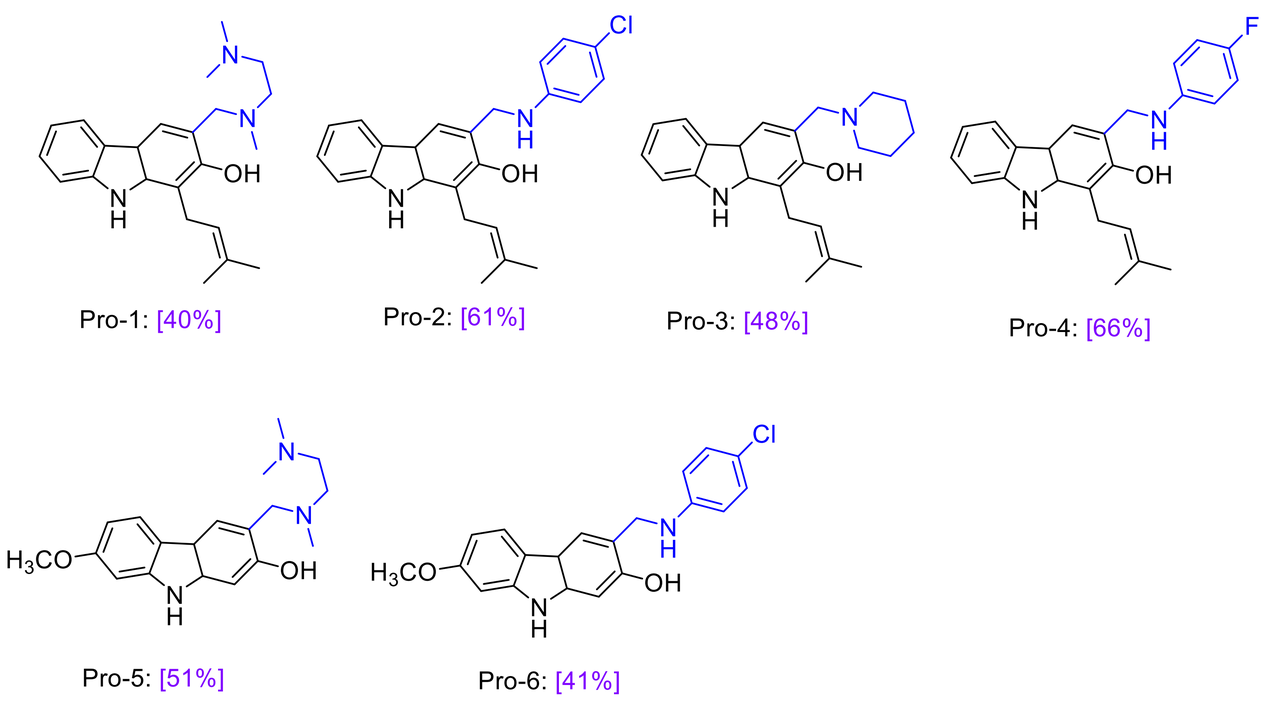


Figure 2 : Library of amino carbazoles derivatives synthetized. Yields are indicated in brackets.

Biological activities:

Yeast screening assay

The activity of a library of amino carbazole alkaloids on several mutant p53 forms with high prevalence in human cancer was investigated using a yeast-based assay developed in previous study [3].

Table 1: Effect of amino heptapylline derivatives compounds at 10μM on the growth of yeast expressing mutant p53 forms with high prevalence in human cancer. Results correspond to the percentage of wild-type p-53 induced growth inhibition reestablished by compounds in yeast expressing mutant p53.

Mutant p53	Pro-1	Pro-2	Pro-3	Pro-4
R280K	X	X	21.1 ± 5.4	X
Y220C	33.3 ± 6.2	X	X	21.3 ± 3.2
G245D	X	X	55.4 ± 9.5	X
R273H	33.2 ± 6.6	X	X	X

Data are mean ± SEM of 4 independent experiments. Crosses represent no significant effect observed.

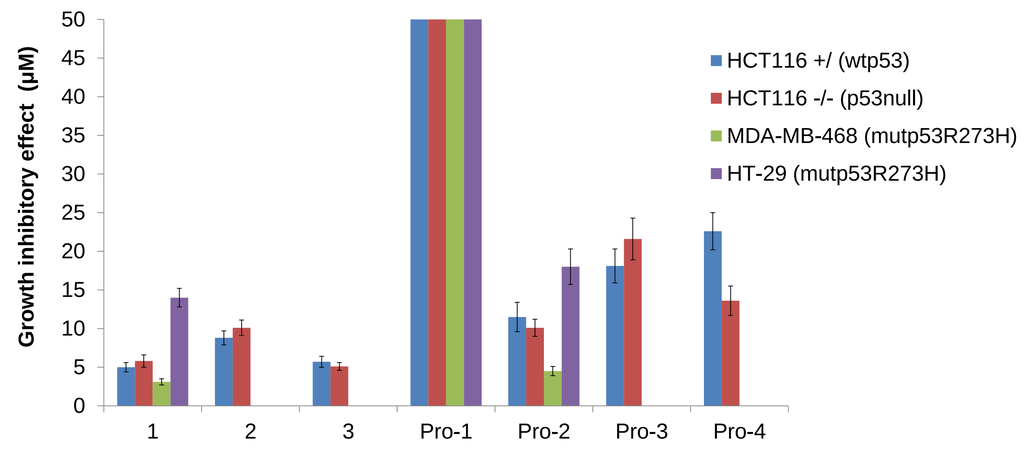


Figure 2: Growth inhibitory effect of heptaphylline derivatives in human tumor cells after 48 h treatment. Data are mean ± SEM of 3 independent experiments. Pro-1 exhibited > 50 μM.

Conclusion

The semisynthesis of carbazole alkaloids was achieved successfully with reductive amination conditions in moderate yields. Natural isolated carbazole alkaloids show potent inhibitory activity on tumor cell growth. Some amino derivatives reestablished the wild-type-like activity to several mutant p53. These results will guide the design and the discovery of a lead compound for cancer treatment by p53 modulation.

References

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